EDGEWOOD
CHEMICAL BIOLOGICAL CENTER

U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND

ECBC-TR-462

STABILITY OF THE INTEGRATED VIRUS DETECTION SYSTEM (IVDS) ELECTROSPRAY MODULE DURING ANALYSIS OF MS2 BACTERIOPHAGE

Charles H. Wick

RESEARCH AND TECHNOLOGY DIRECTORATE

Patrick E. McCubbin

OptiMetrics, Inc.
Bel Air, MD 21015-5203

November 2005

ABERDEEN PROVING GROUND, MD 21010-5424

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
XX-11-2005	Final	Aug 2002 - Mar 2003

4. TITLE AND SUBTITLE
Stability of the Integrated Virus Detection System (IVDS) Electrospray Module During Analysis of MS2 Bacteriophage

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Wick, Charles H. (ECBC); and McCubbin, Patrick E. (OptiMetrics)

5d. PROJECT NUMBER
None

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES)
DIR, ECBC, ATTN: AMSRD-ECB-RT-DD, APG, MD 21010-5424
OptiMetrics, Inc., 2107 Laurel Bush Road, Suite 209, Bel Air, MD 21015-5203

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-462

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
This report characterizes the stability of the electrospray module of the IVDS system while analyzing a sample of MS2 bacteriophage. The electrospray parameters were varied across the stable range, and resultant output from the detector was analyzed.

15. SUBJECT TERMS
MS2, Detection, Virus detection
Virus, Purification, Electrospray stability
Separation, Bacteriophage, Integrated Virus Detection System (IVDS)

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Sandra J. Johnson
U	U	U	UL	13	19b. TELEPHONE NUMBER *(include area code)* (410) 436-2914

Blank

PREFACE

The work described in this report was started in August 2002 and completed in March 2003.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release. Registered users should request additional copies from the Defense Technical Information Center; unregistered users should direct such requests to the National Technical Information Service.

Acknowledgment

Special thanks to Laurie Carey, ECBC; for supplying the initial stock solution of MS2 bacteriophage.

Blank

CONTENTS

FIGURES

TABLE

STABILITY OF THE INTEGRATED VIRUS DETECTION SYSTEM (IVDS) ELECTROSPRAY MODULE DURING ANALYSIS OF MS2 BACTERIOPHAGE

1. INTRODUCTION

Viruses are considered to be among the smallest living particles known to man. Because of their small size, viruses are extremely difficult to detect and characterize. Presently detection and identification of viruses is a complex and expensive biochemical process that requires great expertise.[1] Even new methods that rely on the newest technology are essentially biochemical methods.[2] * The detection process is particularly complex and lengthy when an unknown virus "hits the street." This was illustrated in recent years by the length of time it took to discover and identify the HIV virus.

Recently the U.S. Army Edgewood Chemical Biological Center (ECBC) developed a new system by which viruses are detected and identified by physical rather than biochemical means. This system, the Integrated Virus Detection System (IVDS), relies on the fact that different viruses have different sizes. The system isolates the virus particles from the extraneous material in which they are collected, separates them according to their sizes using a Differential Mobility Analyzer (DMA), and determines their concentrations with a Condensation Particle Counter. The system allows quick screening of many samples at a low cost. The IVDS is further described in ECBC-TR-018.[3]

In this report, we will explore the electrospray injection module of the IVDS system. The electrospray module aerosolizes the virus containing solution and injects a monodispersed aerosol solution into the air stream for analysis. The electrospray converts the sample to an aerosol by charging the liquid with an electric potential, pushing it through a capillary, and exerting an electric field at the capillary tip. The liquid evaporates from the droplets formed at the capillary tip and is carried into the sizing and counting modules of the IVDS.

The parameters associated with the electrospray module are air and CO_2 flow, sample overpressure, electrical voltage, and amperage. The air and CO_2 flows are fixed and are not variable. The overpressure needs to be at a minimum (between 3 and 5 psi) to maintain a flow through the capillary. The only parameter with any variability is the setting of the electrical voltage that exerts the electrical field at the capillary tip. This parameter was varied across the stable range of voltages, and the MS2 bacteriophage was analyzed. Although the amperage changes with voltage, the amperage is not operator adjustable.

The electrical voltage at the capillary tip, which changes the shape of the liquid flow from the tip, was thought to need optimization for repeatable IVDS analyses. This study explores the variation in electrospray voltage and the effects on the IVDS analysis.

*A new silicon chip that harnesses emerging technology at the nano scale will allow the detection of viruses faster, and more accurately, than ever before. Institute of Physics, 20 January 2004.

2. EXPERIMENTAL PROCEDURES

A sample of MS2 stock solution (0.5 mL) was obtained from ECBC. The stock solution was diluted with 50 mL of distilled water and filtered with 50 mL of 20 mM ammonium acetate buffer solution by the ultrafiltration (UF) subsystem of the IVDS using 100K Dalton filter.[4] The filtration system's purpose is to remove any material with a molecular weight smaller than the filtration system is set for (100K Daltons in this case), such as growth media, salt molecules, and proteins from the solution and leave a concentrated virus solution. The filtered MS-2 solution was then diluted at 1 to 10 with the 20 mM ammonium acetate buffer to increase the solutions conductivity to allow it to be injected into the IVDS.

The sample of MS-2 was analyzed by adjusting the electrospray voltage from the minimum voltage that produced a relatively cone to the highest voltage that produced a relatively stable cone. A stable cone would not exhibit any large fluctuations in shape or cause pulsations that would disrupt the cone. Figure 1 shows the range of minimum, optimum, and maximum electrospray cones observed. In addition, image a. in Figure 1 shows the effect of lowering the voltage below the minimum voltage. The electrospray cone produces large drops instead of a stable cone.

The electrospray voltage was ramped in 0.05 kV increments, and three IVDS scans were acquired and saved for each voltage. The amperages were recorded, and the scans were output into MS Excel for analysis. The region of interest (ROI) for the MS-2 is from 22.5 to 27.9 nm. The counts over the ROI were totaled and tabled for analysis.

3. RESULTS AND DISCUSSION

The table lists the kV, nA, sum of ROI, and average and standard deviations of the counts. The counts averaged 1438 counts ± 28 for 12 sets of kV scans. The counts were in very close agreement over the large range of applied voltages. Plotting the kV versus nA in Figure 2 shows the linearity ($R^2 = 0.9942$) of the stable portion of the electrospray cone. The amperage tracks closely with the voltage. Figures 3-6 show the output scans acquired with the IVDS. Each voltage scanned is an average of three individual scans. The scans are in very close proximity to each other, which is verified by the ROI counts obtained from the numerical data files. The change in peak counts was consistent even when the voltages were changed significantly.

4. CONCLUSIONS

This study has shown that there is a wide range of voltage conditions that will produce consistent results. The output count data from the Integrated Virus Detection System (IVDS) for this particular MS-2 sample was very consistent even with the large variation in applied voltage. The electrospray cone, if in a stable configuration, will yield repeatable results from the IVDS.

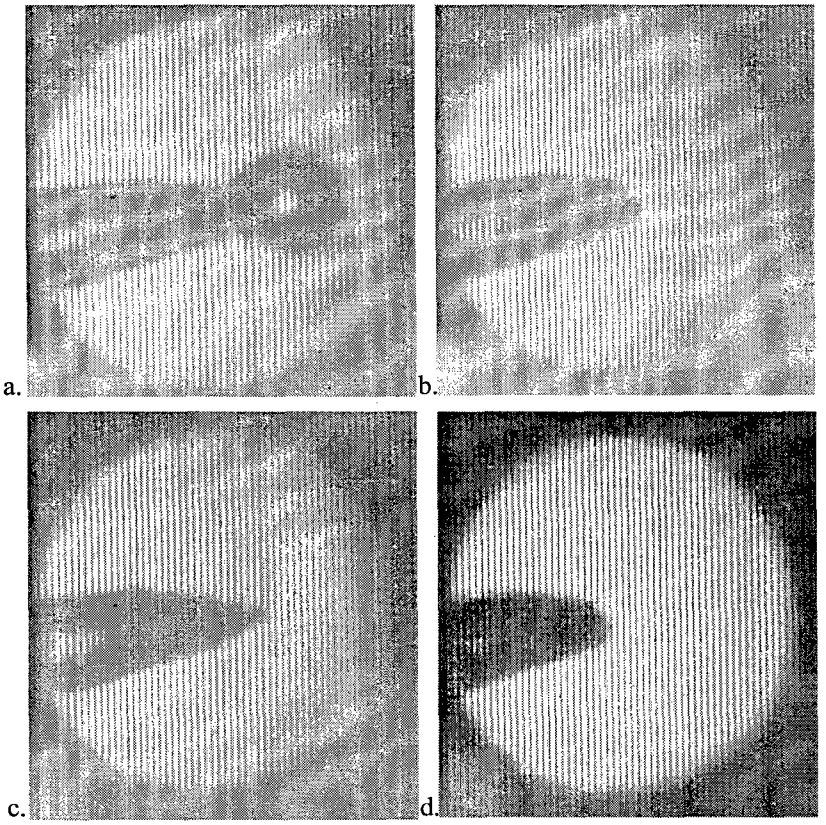

Figure 1. Electrospray Cones. a. dripping, b. minimum voltage, c. optimum voltage, d. maximum voltage

Table. kV, nA, ROI Sums, Average and Standard Deviation of IVDS Scans (Average of 3 scans at each kV setting)

kV	nA	ROI Counts sum 22.5-27.9 nm	
1.75	-228	1457.4	
1.80	-232	1458.5	counts avg
1.85	-235	1454.9	1438
1.90	-238	1408.9	std dev
1.95	-243	1446.1	28
2.00	-249	1430.9	
2.05	-254	1437.6	
2.10	-260	1411.9	
2.15	-264	1392.1	
2.20	-269	1404.9	
2.25	-274	1465.4	
2.30	-280	1483.3	

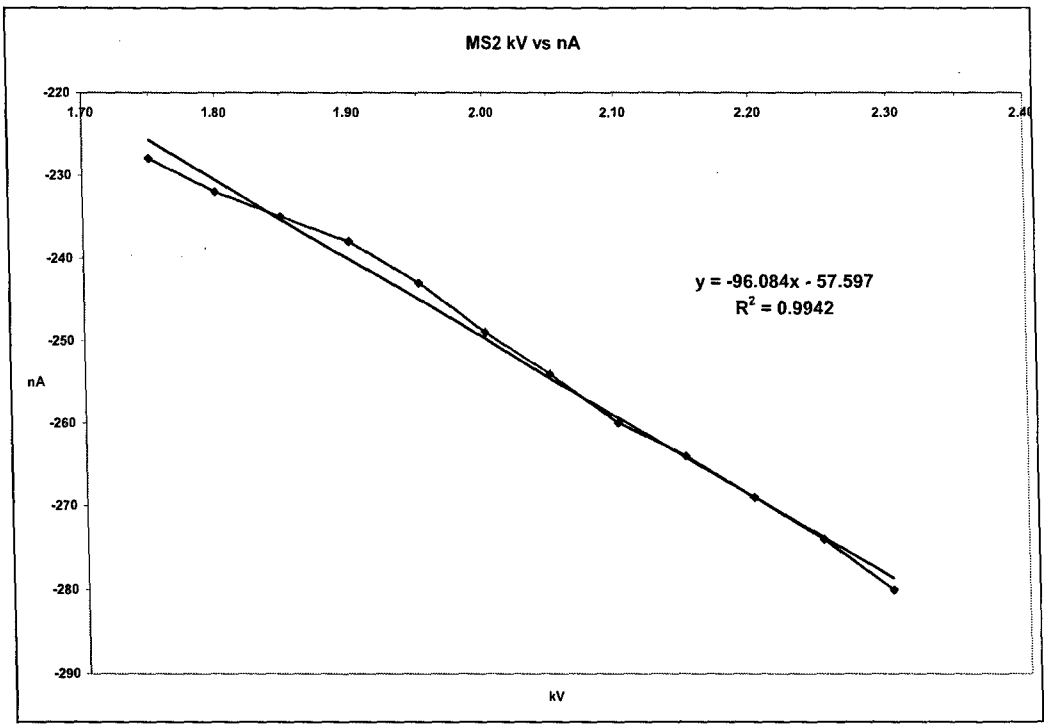

Figure 2. IVDS Electrospray Parameters kV versus nA

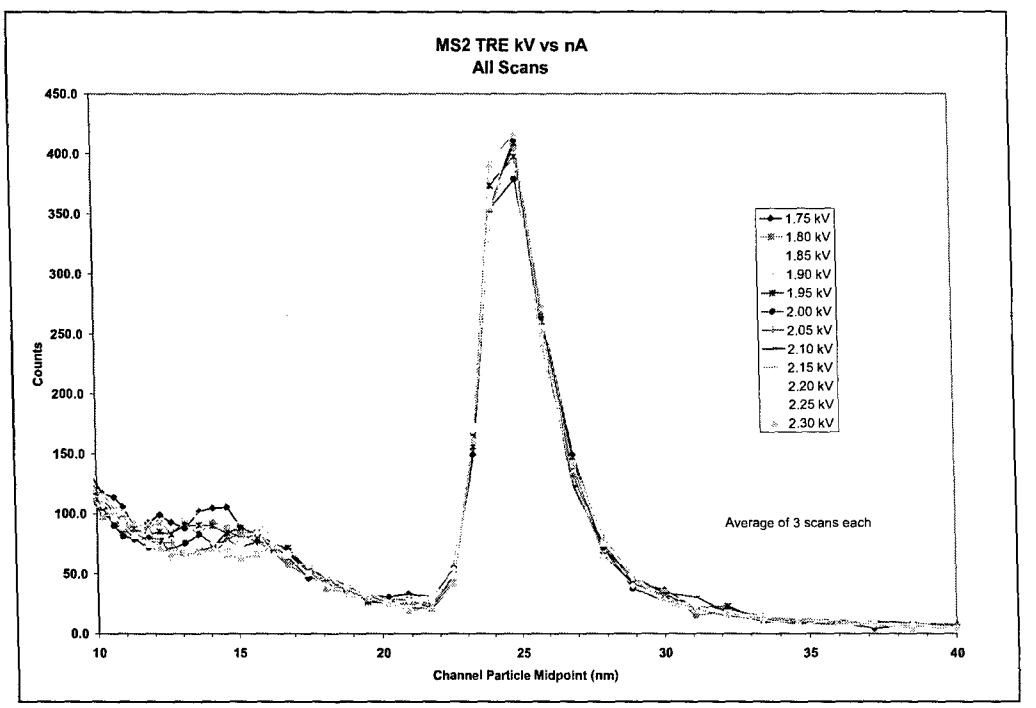

Figure 3. All Scans IVDS kV Adjustment

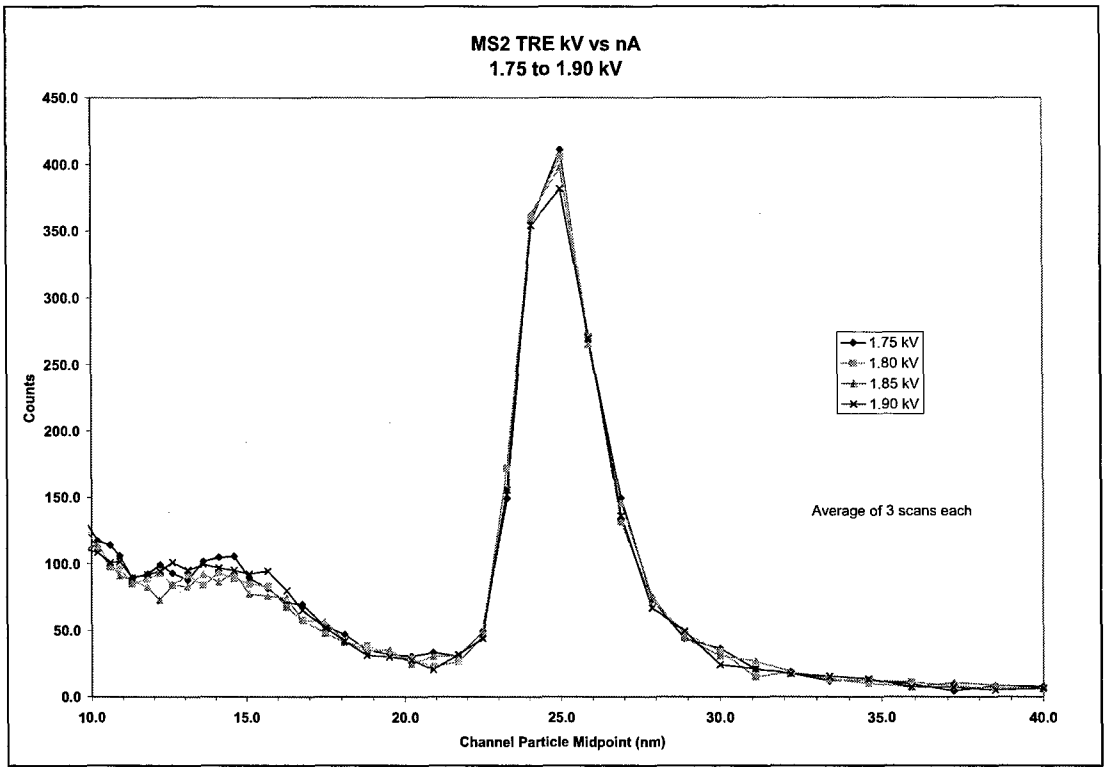

Figure 4. IVDS Scans 1.75 to 1.90 kV

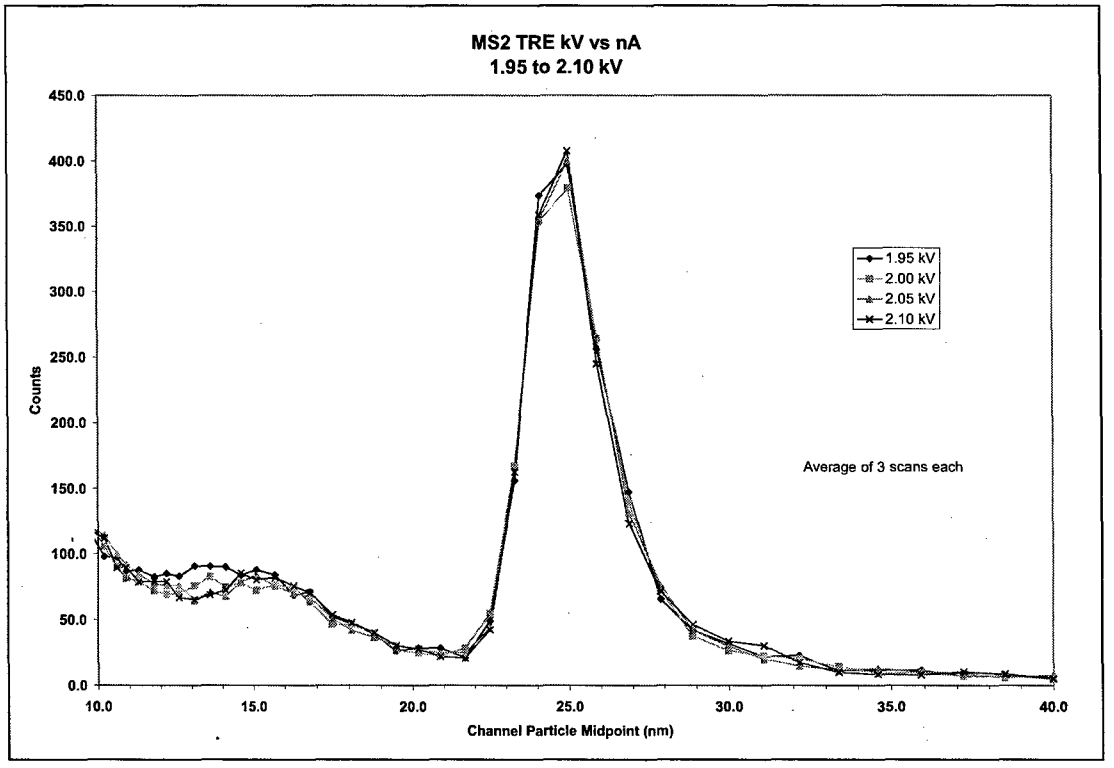

Figure 5. IVDS Scans 1.95 to 2.10 kV

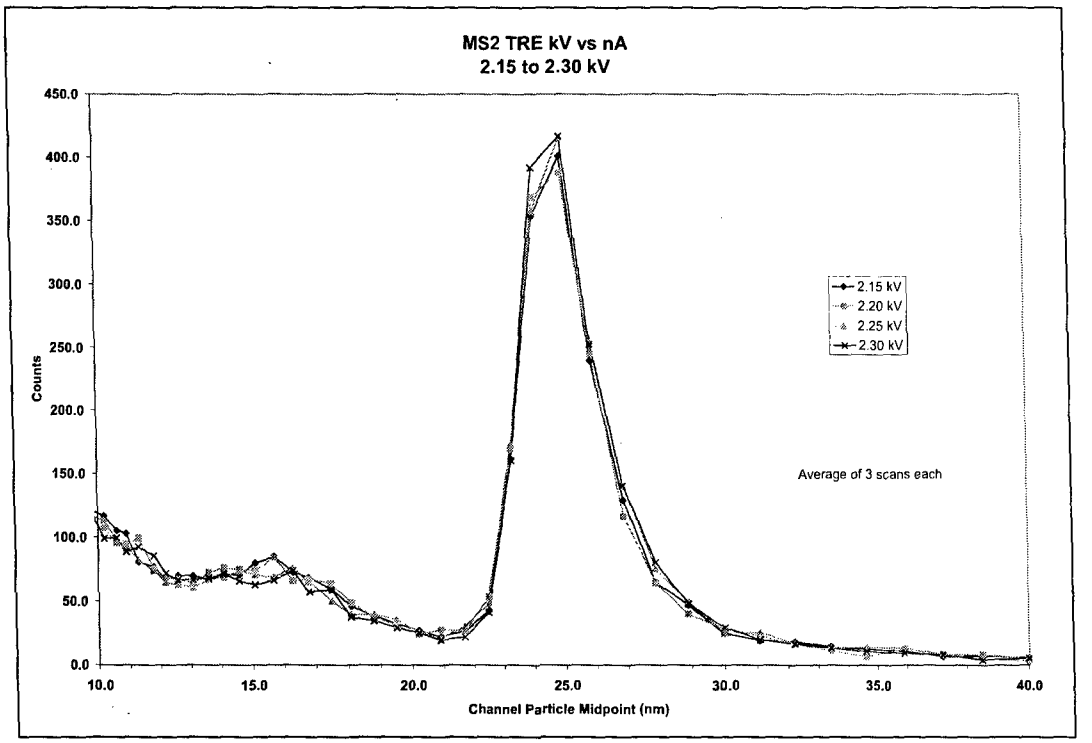

Figure 6. IVDS Scans 2.15 to 2.30 kV

LITERATURE CITED

1. *Optimisation of a New Method for Detection of Viruses in Groundwater*; National Groundwater and Contaminated Land Center, July 2000.

2. http://www8.medica.de/cipp/md_medica/custom/pub/content, lang, 2/ticket, g_a_s_t/oid, 11978/parent, 5965/local_lang (assessed November 2004).

3. Wick, Charles H.; Anderson, David M.; McCubbin, Patrick E. *Characterization of the Integrated Virus Detection System (IVDS) using MS2 Bacteriophage*; ECBC-TR-018; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 1999; UNCLASSIFIED Report (AD-A364 117).

4. Wick, Charles H.; McCubbin, Patrick E.; Birenzvige, A. *Detection and Identification of Viruses Using The Integrated Virus Detection System (IVDS)*; ECBC-TR-463; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2005; UNCLASSIFIED Report.